Preliminary Experiments to Assess the Oncogenicity of Cellular DNA

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Development of Quantitative Assays to Evaluate Potential Risks Associated with Residual Cell-Substrate DNA

Projects Underway in OVRR/CBER to Assess the Risks Associated with Residual Cell-Substrate DNA

- Development of *in vivo* Assays to Assess the Oncogenicity of DNA

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- Development of *in vitro* Assays to Assess the Infectivity of DNA

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Operational Principles for Regulatory Decisions Regarding Cell-Substrate DNA

- Evaluations of risk need to be based on experimental data on the biological activity of DNA
- As human immunization data are unattainable, it is prudent to make estimates based on the <u>most</u> sensitive model systems
- As more data are obtained, risk estimates may change

Development of a Sensitive Animal Model to Assess Oncogenicity of DNA

To develop a quantitative *in vivo* assay to measure the oncogenicity of DNA:

- Choose oncogenes that have been shown to transform efficiently primary cells in culture
- Express these oncogenes under promoters known to function efficiently and for prolonged periods in mice
- Design a “modular” expression plasmid, such that other oncogenes can be evaluated as necessary

Development of a Sensitive Animal Model to Assess Oncogenicity of DNA

Expression Vector

- Promoter is the 5’ MSV LTR
- poly(A) site is from BGH
- Second terminator is the 3’ MSV LTR
- Backbone pLS3 (pUC19 derivative)

Development of a Sensitive Animal Model to Assess Oncogenicity of DNA

Mouse Strains

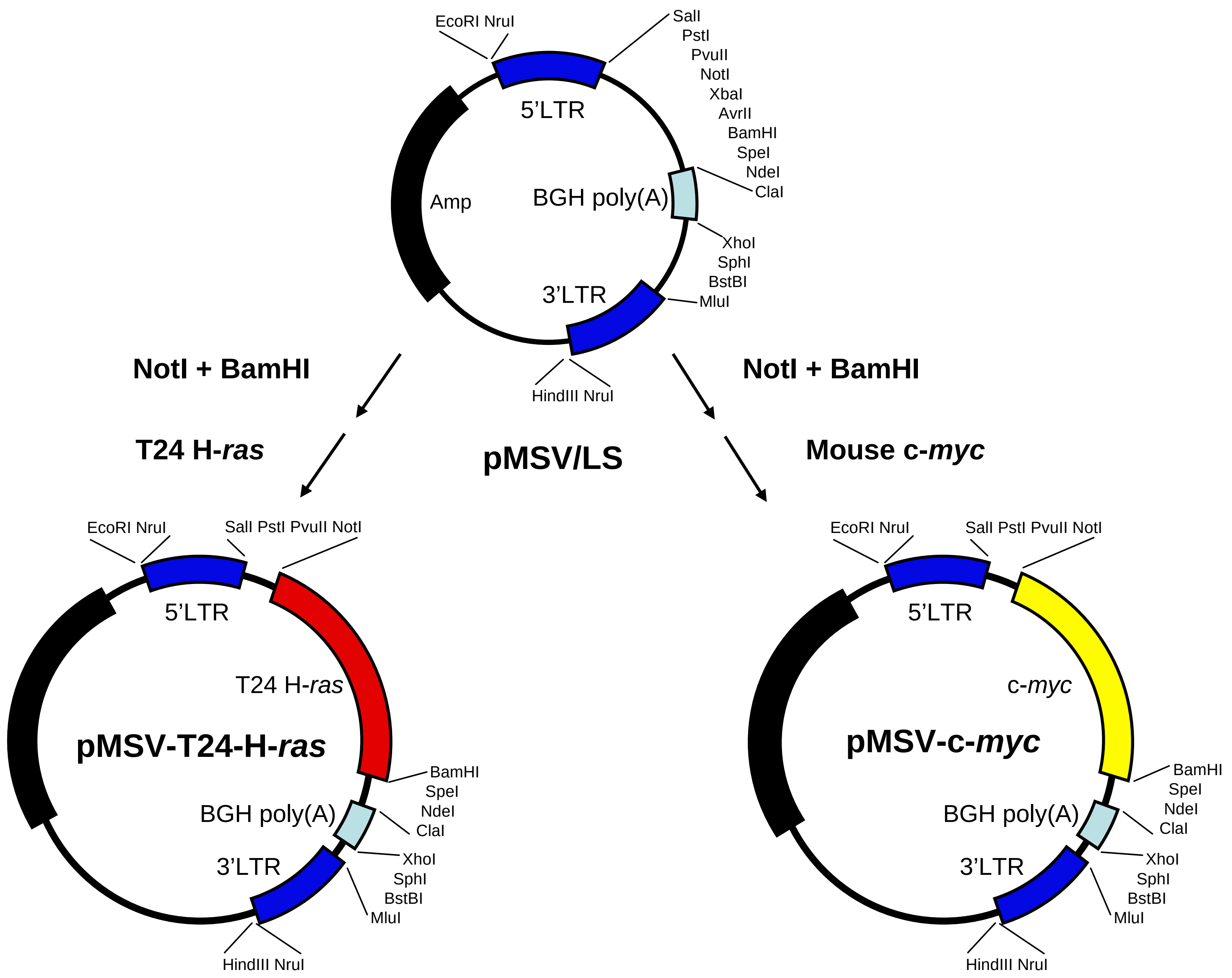
- NIH Swiss
  prototype strain used by Burns *et al*. (1991) for *ras* studies;
  parent strain of athymic nude strain

- Athymic nude (nu/nu)
  T-cell deficient strain

- K6-ODC
  constitutively expresses ODC under the keratin 6 promoter;
  chronic state of tumor "promotional" stimulation

- C57BL/6
  parent strain of K6-ODC mice

Construction of pMSV-T24-H-*ras* and pMSV-c-*myc*

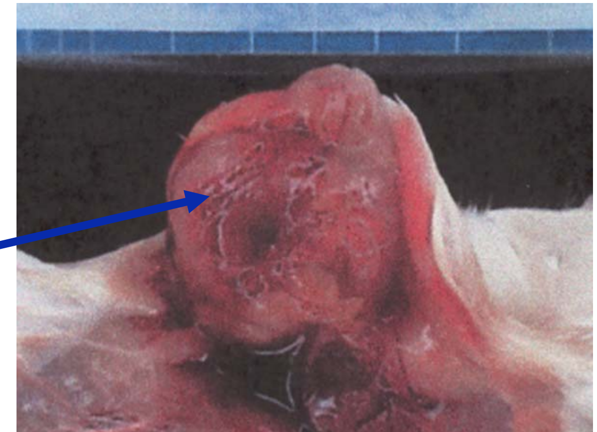


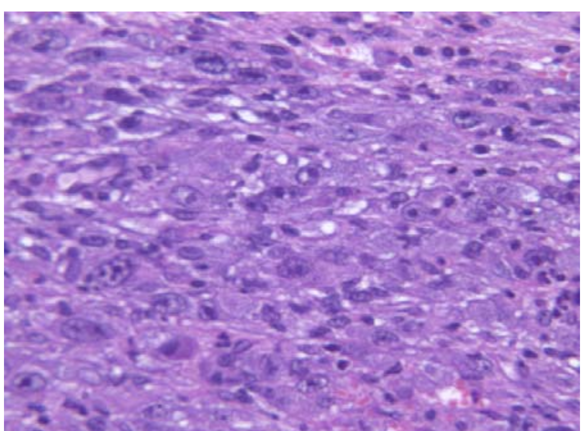
Tumor Incidence in Mice Injected with *ras* and *myc* Expression Plasmids

Cohort	*ras* Plasmid (µg/mouse)	*myc* Plasmid (µg/mouse)	Swiss		C57BL/6	
			Adult	Newborn	Adult	Newborn
R6	12.5	0	0/10	0/10	0/10	0/8
R5	1.25	0	0/10	0/11	0/10	0/10
R4	0.125	0	0/10	0/9	0/10	0/10
R3	0.0125	0	0/10	0/8	0/10	0/10
RM6	12.5	12.5	**2/10**	**9/11**	**0/10**	**2/11**
RM5	1.25	1.25	0/10	0/10	0/10	0/10
RM4	0.125	0.125	0/10	0/10	0/10	0/10
RM3	0.0125	0.0125	0/10	0/10	0/10	0/10

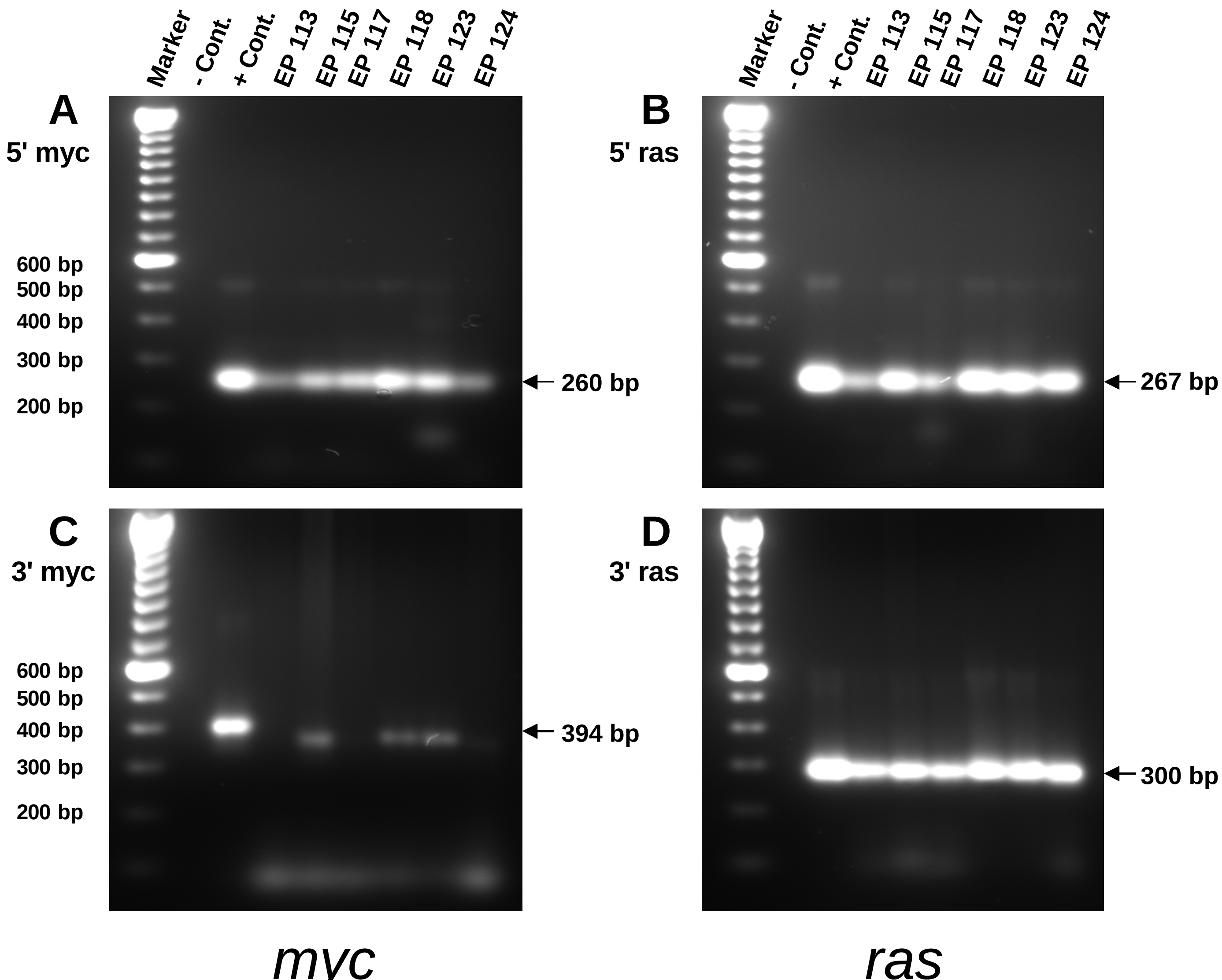
Sub-cutaneous sarcomas developed at injection site at 4 - 7 weeks

ras-myc Tumor in NIH Swiss Mouse





PCR Analysis of *ras* and *myc* Genes in Tumor Cell Lines



Summary of the Results: Oncogenicity of DNA

- An MSV LTR expression plasmid has been constructed and tested *in vitro* for activity (luciferase)
- Expression plasmids for T24 H-*ras* and murine c-*myc* under the MSV promoter have been generated and shown to be active in *in vitro* transformation assays
- The *ras* and *myc* plasmids are able to induce tumors in normal mice: NIH Swiss > C57BL/6; newborn > adult
- Cell lines from tumors have *ras* and *myc* DNA

An *in vitro* Assay to Assess DNA Infectivity

Li Sheng and Keith Peden
CBER/FDA

Cloned HIV plasmid transfected into 293T cells

Co-culture with Jurkat cells

Follow infectivity with reverse transcriptase activity in medium

Calculations of Risk Based on DNA Infectivity - 1
Extrapolations from Viral-Infected Cellular DNA

DNA from HIV-infected cells was tested for infectivity

2.5 µg of this DNA was infectious (in 2 out of 4 experiments; 5 µg in 4 out of 4 experiments)

Assume that between 1 and 2.5 µg infected cellular DNA is infectious

Therefore, in the <u>absence</u> of any treatment (chemical, enzymatic, radiation),residual cell-substrate DNA at **10 ng** could have a safety margin of **100 - 250 fold**

Calculations of Risk Based on DNA Infectivity - 2

Extrapolations from Infectivity of Cloned HIV DNA

Cloned HIV DNA is infectious at 1 pg

HIV proviral genome represents 1.67×10^{-6} of the diploid cell genome

Therefore, the amount of cellular DNA that would correspond to an infectious HIV dose is:

$$1 \text{ pg} \div 1.67 \times 10^{-6}$$

0.6 µg (600 ng)

Thus, in the absence of treatment (chemical, enzymatic, radiation), **10 ng** of cellular DNA provides a **60-fold** safety factor

Calculations of Risk Based on DNA Infectivity - 3
Digestion with Omnicleave Nuclease

Digestion of DNA to a mean size of 650 bp resulted in the loss of biological activity of 0.15 µg of pure viral DNA

Based on the proportion of a retroviral genome in the cell, 150 ng of viral DNA corresponds to:

$$150 \div 1.67 \times 10^{-6} \text{ of cellular DNA}$$
$$= 90 \times 10^{6} \text{ ng}$$
$$= 90 \text{ mg}$$

Therefore, for 10 ng of cellular DNA with a single provirus, the safety factor is:

$$9 \times 10^{6}$$

Calculations of Risk Based on DNA Infectivity - 4

Inactivation with β-Propiolactone

Treatment of cloned HIV DNA with 0.25% β-propiolactone (BPL) for 72 h at 4°C eliminated the activity of 50 ng

Based on the proportion of a retroviral genome in the cell, 50 ng of viral DNA corresponds to:

$$50 \div 1.67 \times 10^{-6} \text{ of cellular DNA}$$
$$= 30 \times 10^{6} \text{ ng}$$
$$= 30 \text{ mg}$$

Therefore, for 10 ng of cellular DNA with a single provirus, the safety factor is:

$$3 \times 10^{6}$$

Conclusions from Calculations of Risk Based on DNA Infectivity Studies

- DNA infectivity with HIV-infected cellular DNA and cloned HIV DNA gave similar safety factors for 10 ng DNA
  100 - 250 fold with cell DNA, 60 fold with cloned DNA
  Therefore, the infectivity of a retroviral genome is similar whether integrated or not
- With 10 ng of cellular DNA, nuclease digestion to a mean size of 650 bp could provide a safety margin of 9×10^6
- With 10 ng of cellular DNA, treatment with BPL could provide a safety margin of 3×10^6

These studies suggest ways of obtaining a $>10^7$ fold margin of safety with respect to residual cell-substrate DNA.
i.e., reducing the size of the DNA or inactivating the DNA and having <10 ng DNA per dose.